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SHORT COMMUNICATION

THE EFFECTS OF APPLIED HORMONES ON GERMINATION OF EXCISED EMBRYOS OF *PROTEA COMPACTA* R.Br. IN VITRO*

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ABSTRACT

It is suggested that the inhibitor(s) present in the embryos of *Protea compacta* block both chlorophyll synthesis and germination. The latter process appears to be more effectively inhibited than the former. Kinetin and gibberellic acid can partly overcome the inhibition of both these phenomena while indoleacetic acid can only overcome the inhibition of chlorophyll synthesis.

UITTREKSEL

DIE INVLOED VAN TOEGEDIENDE HORMONE OP DIE ONTKIENING VAN *PROTEA COMPACTA* R.Br. IN VITRO.

Daar word voorgestel dat die inhibeerder(s) in *Protea compacta* embrios beide bladgroensintese en ontkieming inhibeer. Laasgenoemde word skynbaar meer effektief gestrem as eersgenoemde. Kinetien en gibberelliensuur kan die inhibisie van beide prosesse gedeeltelik oorkom terwyl indoolasynsuur slegs effektief is in die geval van chlorofilsintese.

It has previously been reported (Atkinson, 1961; Brown and Van Staden, 1971) that excised embryos of certain proteaceous species did not resume normal growth when placed under conditions suitable for germination. When excised, the embryos were very susceptible to fungal infection. Treatment with a fungicide, however, did not improve germination. The fact that removal of the testa did not increase germination suggested that dormancy was not coat imposed, but rather due to innate embryo dormancy. This is possibly due to inhibitors which have been found in the embryo extracts of a number of species (Van Staden and Brown, 1972) or to a lack of promotors.

Wareing and Saunders (1971) reported that the embryo dormancy in a wide variety of seeds can be overcome by the application of gibberellins and cytokinins. In this study gibberellic acid (GA_3), indoleacetic acid (IAA) and kinetin were applied to excised embryos of *P. compacta* under sterile conditions in an attempt to overcome dormancy. Excised embryos were used as it was considered that the hard seed coat, enclosing the mature embryos would severely restrict the movement of applied growth regulators.

Seeds were sterilized by immersion in 3,5% sodium hypochlorite solution for 15 min. followed by 5 min. in 1% Cetavlon in 95% ethanol. They were then

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rinsed in sterile water, the seed coats removed, and the embryos transferred to the culture tubes. Each treatment was replicated 12 times.

The embryos (cotyledons plus radicles) were cultured on the medium of Murashige and Skoog (1962) to which was added 2% sucrose and 1% agar. Each of the following concentrations (mg/l) of growth regulator was tested separately: Kinetin and IAA (0,0001; 0,01; 0,1; 1,0; 10,0) and GA_3 (0,01; 0,1; 1,0; 10,0; 100,0). Cultures were kept at 25° with 12 hr photoperiod (light intensity $3,0 \times 10^2$ Im/m^2). Elongation of the radicle was regarded as positive evidence of germination.

The first visible change to the naturally etiolated embryos was the appearance of chlorophyll in the cotyledons. This greening of the cotyledons always preceded radicle growth (germination) where the latter occurred. In most cases, however, greening of the cotyledons was not followed by radicle growth (Table 1). This suggests that the block to chlorophyll synthesis can be overcome more easily than the block to radicle growth. Alternatively it is possible that two different inhibitors are functional in the embryos, the effect of one being more easily overcome than that of the other. It is quite possible that this inhibition of chlorophyll synthesis in excised *P. compacta* embryos is a result of the inhibition of protein synthesis as Bogorad (1966) has shown that continuous synthesis of proteins is necessary for the maturation of proplastids to chloroplasts in etiolated leaves.

The application of IAA did not bring about germination of excised embryos. It would appear, therefore, that IAA is unable to overcome the inhibition of radicle growth. The number of embryos in which chlorophyll appeared, however, increased five-fold by the application of 0,1 mg/l IAA. With an increase in GA_3 in the culture medium from 0,01—1,0 mg/l the percentage of embryos in which chlorophyll synthesis occurred, increased from 25—57. Germination, however, only occurred when 1,0 mg/l GA_3 was applied. This concentration of GA_3 resulted in 43% of the embryos germinating compared to the 21% of intact seeds in petri dishes. Higher concentrations of GA_3 (10 and 100 mg/l) did not bring about any growth. Although not as effective as GA_3, a concentration of 0,01 mg/l kinetin resulted in increased germination of the excised embryos. The apparent inability of 0,1 mg/l of kinetin to enhance greening or germination cannot be explained. At a concentration of 1,0 mg/l kinetin considerable elongation of the radicle occurred, but no new roots were formed. This was probably due to the fact that the concentration of kinetin in the medium was too high for root formation.

Van Staden and Brown (1972) found a number of germination inhibitors in extracts of dormant *P. compacta* seed. The major inhibitor appeared to be coumarin-like in its properties. Whether or not these inhibitors play a role in the dormancy of the seed under natural conditions was not shown.

In situations where coumarin has been known to inhibit germination, the effect cannot be reversed by IAA or GA_3 (Khan and Tolbert, 1966). Knypl (1967) reported that GA_3 was only effective in overcoming the effect of coumarin in kale seeds after prolonged periods of soaking. However, he found that kinetin could markedly reduce its inhibitory effect. There thus may be an interaction between coumarin, GA_3 and kinetin similar to the one existing between abscisic acid (ABA), GA_3 and kinetin in situations where ABA is responsible for seed dormancy.

The present data do not contribute to our knowledge of the nature of the inhibitor in dormant embryos of *P. compacta* beyond suggesting that there may be an interplay between it, GA_3 and/or kinetin.

Webb and Dumbroff (1969), Corcoran (1970) and Thompson (1970) have shown that embryo dormancy of a number of species can be overcome by applying gibberellins and cytokinins to intact seed. The possibility of breaking the dormancy of proteaceous seed in a similar way is at present being investigated.

TABLE 1.
Effects of kinetin, IAA, and GA_3 on the development of *Protea compacta* embryos in culture

Hormone	Treatment Concentration (mg/l)	% Cotyledons Green	% Germination
Control	—	10	0
Kinetin	0,001	50	0
	0,01	50	33
	0,1	0	0
	1,0	57	14
	10,0	0	0
IAA	0,001	0	0
	0,01	25	0
	0,1	50	0
	1,0	0	0
	10,0	0	0
GA_3	0,01	25	0
	0,1	43	0
	1,0	57	43
	10,0	0	0
	100,0	0	0
Intact seed in petri dishes: 21% germination			

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